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GERMINATION OF SPORES AND DE-
VELOPMENT OF JUVENILE THALLUS
OF *RIELLA AMERICANA*

A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

By
RICHARD ARTHUR STUDHALTER

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CHICAGO, ILLINOIS

Reprinted from
THE BOTANICAL GAZETTE, Vol. XCH, No. 2, 1931



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GERMINATION OF SPORES AND DEVELOPMENT OF JUVENILE THALLUS OF *RIELLA AMERICANA*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 420

R. A. STUDHALTER

(WITH TWENTY-FIVE FIGURES)

Introduction

Botanists have long been interested in *Riella*, a genus of submerged aquatic liverworts, because of its peculiar shape. The adult plant consists of a thickened stem, on one side of and over the tip of which is a wing one cell in thickness. Rhizoids develop near the base of the stem; archegonia are produced along its margin; and antheridia develop along the outer edge of the wing, which is here somewhat thickened. The origin and morphological interpretation of the wing have been subjects of much controversy.

The first known species of *Riella* was called *Sphaerocarpus notarisii*, and was described from Sardinia in 1839. With the discovery of a new form in Algeria, the two species were placed together into the new genus *Duriacea* in 1843. In 1852, MONTAGNE added a third species from Switzerland to the group, and changed the name to *Riella* on account of the previous use of the name *Duriacea* for a genus of the Umbelliferae. Eleven species are now known, from Algeria, Sardinia and Greece, Switzerland, France, Turkestan, South Africa, the Canary Islands, and Texas. The distribution of each is more or less local, some having been collected only a few times; and one species has not been seen in its native habitat, having been seen only in culture from mud collected in Turkestan (11).

Riella americana was described in 1903 by HOWE and UNDERWOOD (9) from specimens collected in western Texas. This, together with a fragmentary specimen from the same place in 1855, and another fragmentary specimen from South Dakota in 1898, are the only recorded collections of the species until its rediscovery by the writer

in the spring of 1927. The work of HOWE and UNDERWOOD is apparently the only published paper on the species.

Materials and methods

The spores used in the germination studies herein reported were obtained in western Texas at various times from 1928 to 1930. Plants with mature sporophytes were collected, together with some of the sand, from the bottom of the creek in which they were growing. In most instances this mixture was permitted to air-dry in the laboratory, but in other cases it was kept in water in a closed jar until used.

For purposes of germination, some of this material was placed during the warmer months of the year in a moist chamber or jar in 2-3 inches of distilled water and placed on window sills where direct sunlight was available part of the day. In other instances the cultures were heated to temperatures ranging from 40° to 75° C. for one or two hours before being placed in the window. The evaporated water was replaced with distilled water, and occasionally a mixture of distilled and tap water. The presence of the sand from the natural habitat is believed to have furnished a partially natural condition for the material.

For the purpose of study, some of the sediment in the bottom of the culture was drawn off with a wide mouthed pipette. Every few days a portion of the material was preserved in formalin-alcohol for future comparison with the fresh green sporelings, the latter serving as the basis for most of the study as well as for the drawings.

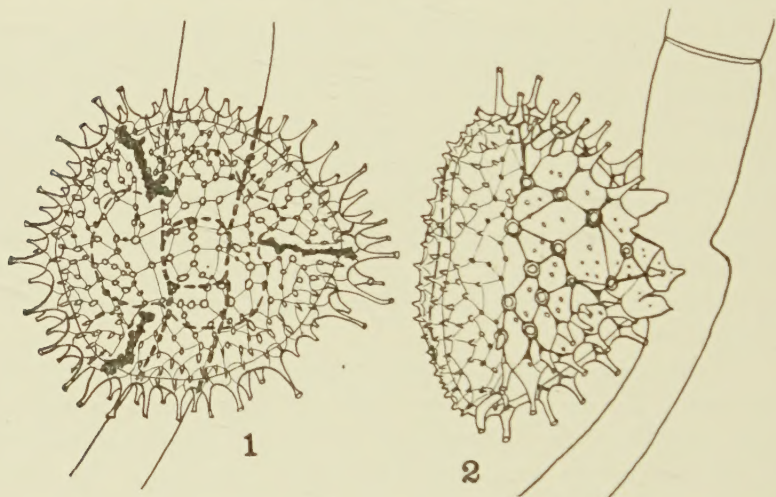
The more elaborate methods used by other workers in germinating the spores of *Riella*, often in addition to simple water cultures, were not found necessary for *R. americana*. PORSILD (12) used mud, filter paper, loess, or kaolin covered with water. HOWE and UNDERWOOD (9) used filter paper. GOEBEL (6) also used kaolin. It is believed that pipetting the sporelings is less injurious to them than picking them off of a more solid substrate; but this method has the disadvantage of making it more difficult to obtain a clear idea of the orientation of the sporelings in the water, and of disturbing this orientation more frequently.

First germination stages

The spores of *Riella americana* are described by HOWE and UNDERWOOD (9) as follows:

dark-brown, 100-130 μ in maximum diameter (spines included); outer face bearing numerous sometimes curved spines 10-24 μ long, with dilated apices, these spines more or less connected by radiating basal membranes forming irregular reticulations; inner faces bearing conical, non-capitate spines, 3-6 μ long, with basal membranes obsolescent or entirely wanting.

As the spore matures, the outer face becomes somewhat conical and the inner face flattened or even depressed at its middle. The



FIGS. 1, 2.—Fig. 1, germinating spore from inner face; long capitate spines of outer face extend over margin; irregular radial ridges shown in solid black, boundary of central depression dotted; fig. 2, germinating spore from side; germ tube emerging from middle of outer and more spiny face; $\times 420$.

triradial ridges are usually completely obliterated, and, if seen at all, are merely rough, irregular, dark ridges toward the outer portion of the inner face (figs. 1, 2).

The spores of *R. americana* are found to germinate better after a period of rest. Fresh spores, apparently mature, give a low percentage of germination; while spores from the same material up to 17 months of age (the oldest available) which had been kept dry or in water in the dark, germinated in excess of 50 per cent in a few

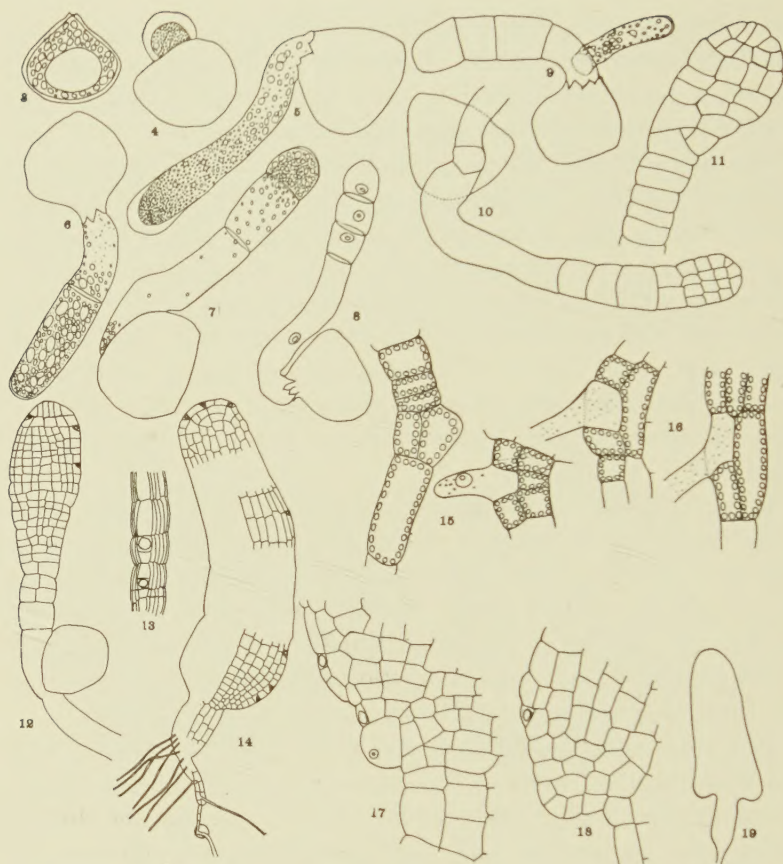
days. Heating the spores in water before the culture was made was found to hasten germination of air-dried spores recently collected, but often to be detrimental to older spores. Strong sunlight was found advantageous in the early stages of germination.

The spores of other species of *Riella* also require a rest period after apparent maturity. Germination resulted for HOWE and UNDERWOOD working with *R. americana* in the case of only a few of the spores collected several months previously. The same workers obtained more than 50 per cent germination of spores of *R. affinis* in a few days from specimens which had been in the herbarium for five and a half years. Spores of *R. paulsenii* germinated for PORSILD (11, 12) from mud collected about three years previously, and CAVERS (2) obtained germination of *R. capensis* from dried mud six years old.

In 1912, Dr. C. J. CHAMBERLAIN received from South Africa some dry soil from which a culture was made. Algae developed but no *Riella* made its appearance. *R. capensis* appeared very abundantly in a second culture, however, made from the same soil in 1925, 13 years after collection. Hundreds of plants were carried to the fruiting stage. This information was transmitted verbally by Dr. CHAMBERLAIN, with permission to quote it.

In several instances intracapsular germination was observed for the Texas species. Many of the spores germinated simultaneously, the germ tubes and rhizoids readily breaking through the slightly decomposed sporangium wall. Such germination was also seen by PORSILD (12) for *R. paulsenii*, and is mentioned by DOUIN (4) and GOEBEL (7).

There is little or no swelling of the spore of *R. americana* during germination; it remains essentially the same size and shape, and the depression at the middle of the inner face does not straighten or bulge out (fig. 2). Within two or three days after dried spores are placed under conditions favorable for germination, the wall becomes slightly more transparent and oil drops of various sizes are seen to occupy a large part of the interior of the spore (fig. 3). Just before rupture of the spore wall, a single large vacuole is formed opposite the middle of the inner face (fig. 3). The nucleus is not visible in unstained spores. The time required for the rupture varies from 4



FIGS. 3-19.—Fig. 3, outline of spore with wall about to rupture; fig. 4, germ tube emerging from under side of spore; fig. 5, sporeling before formation of first cross wall; fig. 6, sporeling after formation of first cross wall; fig. 7, after formation of second cross wall (all $\times 165$). Fig. 8, sporeling after formation of third cross wall, showing positions of nuclei; $\times 140$. Fig. 9, sporeling showing origin of primary rhizoid; distal cell slightly green, other two yellowish; fig. 10, division of distal cells to form early stage of primary thallus; all cells green; primary rhizoid three times length of germ tube; fig. 11, further divisions of distal cells of filament; $\times 165$. Fig. 12, later stage of primary thallus; $\times 115$. Fig. 13, portion of primary thallus seen from edge, with two large oil cells; $\times 165$. Fig. 14, mature primary thallus; $\times 75$. Fig. 15, two stages of origin of secondary rhizoid; fig. 16, two figures showing relation of secondary rhizoid to mother cell and surrounding cells; $\times 165$. Fig. 17, intercalary apical cell at base of expanded portion of primary thallus; fig. 18, early stage of lateral intercalary outgrowth; $\times 165$. Fig. 19, primary thallus showing lateral outgrowth on both sides; twin plants result in such cases; $\times 37$.

to 18 days, the shorter periods being more common. The rupture takes place at the middle of the outer or more spiny face (fig. 2), which is the apex of a rounded pyramid. This point of rupture, previously reported by HOWE and UNDERWOOD, is the exception for the spores of bryophytes and pteridophytes, which usually germinate at the middle of the inner face, that is, at the junction of the radial cracks or ridges. Possibly this condition in *R. americana* is associated with the shape of the spore, the apex of the pyramid on the outer face representing the weakest part of the spore wall. Or, possibly it is a hold-over from an ancestral condition in which the spores of a tetrad cling together after maturity, as do those of *Sphaerocarpus* (CAMPBELL 1). The rupture is effected by portions of the spore wall being lifted away from the apex and raised more or less at right angles to the spore. The resulting opening is characteristically circular (fig. 1); occasionally triangular, square, or irregularly angled openings are found, but always with the two dimensions roughly equal.

The first structure to break through is the germ tube (fig. 4), although in rare instances the primary rhizoid makes its appearance first. The germ tube at first grows out straight from the spore, but usually before it reaches a distance of 100 μ it bends to one side (fig. 5). This is no doubt a reaction to the stimulus of light or gravity, for occasionally growth is straight outward, and at other times the tube follows the spore wall half way around the spore before growing away from it. The end wall of the tube is at first somewhat thick (fig. 4), but later is no thicker than the side walls. The germ tube reaches a length of 80–150 μ before the first cross wall is formed (fig. 6); under conditions of weak illumination a length of 500 or even 700 μ has been observed. The diameter of the germ tube is nearly uniform throughout, ranging from 26 to 44 μ with an average of 33 μ . Generally, however, it is somewhat narrow at the spore and slightly enlarged at the tip.

The contents of the germ tube consist largely of closely packed oil drops (fig. 6). Small granules are also visible. As the germ tube grows, the oil drops become smaller and more scattered, remaining most numerous toward the tip of the tube. Chloroplasts and rhizoids are not seen at this stage.

HOWE and UNDERWOOD were the first to report on the early stages of germination of any species of *Riella*. In both *R. americana* and *R. affinis* they found the germ tube emerging before the rhizoid; in the former species it always developed from the outer face near its middle, and in the latter the same was true in practically all cases. The shape of the opening in the spore wall is not described nor shown in the drawings. PORSILD (12) found that the spore of *R. paulsenii* was cracked open along a straight median line more than half way around the spore. He found that a simple germ tube was formed, the length and width of which are dependent upon light, being short and broad in well lighted cultures on filter paper or kaolin. DOUIN (4) shows, without comment, a sporeling of *R. clausonis* in which the spore is split open along a straight median line. Other investigators give no details whatever on this stage, or even fail to mention it.

Filamentous stage

At the time of formation of the first cross wall, 45–115 μ from the spore, the oil drops are more numerous toward the tip of the filament, and no chloroplasts are visible. Growth of the filament continues at its tip, which remains rounded, and soon a second cross wall is formed (fig. 7) about midway between the first one and the tip. Growth continues in this manner until four to seven cells are formed in a linear series (fig. 8). These cells have an average length of 35 μ and an average diameter of 33 μ . Even in those cases in which the filament had become enormously elongated before being divided by cross walls, the new cells formed were of the sizes indicated.

As growth continues, the oil drops become smaller and gradually disappear; chloroplasts make their appearance, taking their place (fig. 9). The plastids are sometimes found in the 1-celled stage, but more frequently are not seen until two or three cross walls have been formed. The terminal cell is the first to become yellow, and then green, and the greening proceeds cell by cell toward the spore. In no case were plastids seen between the spore and the nearest cross wall, this region of the germ tube remaining colorless and containing scattered small oil drops and granules.

The primary rhizoid is generally formed after the second or third cross wall has been laid down (fig. 9). It makes its appearance on

the convex side of the germ tube, between the spore and the nearest cross wall, close to or directly at the spore. From a circular area a globular protrusion is formed, somewhat smaller in diameter than the germ tube, and the rhizoid thus formed protrudes farther as a tubelike outgrowth, which bends back over the spore and grows in a direction opposite to that of the germ tube. It is a cylindrical, smooth walled structure of uniform diameter ($17-28\ \mu$, average $20\ \mu$), with a rounded, thickened end wall. Its lumen is continuous with that of the base of the germ tube. Only in rare instances was a single cross wall observed, always some distance from the spore. The protoplasm remains granular and is at first densely crowded with oil drops, which become scattered as the rhizoid increases in length, but which remain more numerous near the tip. The nucleus is found a short distance back from the tip.

The primary rhizoid elongates rapidly, while the filament slackens its rate of growth so that the rhizoid is soon much longer than the germ tube. No secondary rhizoids have been observed at this stage. The growth of this stage is completed in two or three days.

HOFMEISTER (8) states that in *Riella reuteri* a short filament is formed upon germination, but he presents no description nor figures. TRABUT (16) says nothing about the early stages of development of *R. cossoniana*, but he shows two figures (without indicating cells) which he calls the protonema, but which, as pointed out by HOWE and UNDERWOOD, are more likely plants originating from gemmae. HOWE and UNDERWOOD state that in *R. americana* the germ tube is divided by transverse walls into a single row of several cells. More detail is given by these writers for *R. affinis*, in which the first wall was found to be somewhat curved, with its convexity turned toward the spore. The part above contained most of the starch grains, and in the course of time began to show chlorophyll. The length of the germ tube from the spore wall to the curved septum was found to vary from 0.02 to 0.7 mm. SOLMS (13) states merely that a filament of cells is produced by *R. parisii*. PORSILD (12) found that in *R. paulsenii* the germ tube becomes divided immediately by cross walls, and that the first rhizoid usually develops at the first cross wall.

Primary thallus stage

A few days after germination, when the filament has reached four to seven cells in length, its end broadens and becomes rounder. The exact order of wall formation was not observed, but in a short time the end cell has divided longitudinally and transversely into several small cells, all in the same plane, forming a rounded flattened structure one cell in thickness (fig. 10). Soon the second cell from the tip undergoes similar divisions, then the third, etc., in basipetal succession (fig. 11). The result is that the cells are smaller and younger at the tip, and progressively larger and older toward the spore. All oil drops disappear, and the peripheral regions of each cell become closely packed with chloroplasts.

For a time the derivatives of each of the original cells can be followed distinctly (fig. 12). The new thallus is at first broader at its tip, and later broader at or just below its middle. The thallus at this stage is usually less than 1 mm. in length at its maximum, but one thallus 1.5 mm. long was observed. Its width varies up to 0.5 mm. (usually less), and up to 30 cells at its widest part. Its shape varies from almost circular to egg-shaped, elongated oval, or much elongated. Division is in two planes, so that the thallus remains one cell in thickness, although exceptions have been found rarely in which the middle basal region is two cells in thickness. The thallus is somewhat lens-shaped in cross-section; the marginal cells are 13-18 μ thick, and the cells become progressively thicker toward the center of the thallus, where they have the thickness of the original cells of the filament, 35 μ (fig. 13).

No apical cell has been observed in the thallus at this stage. The cells at the tip remain smaller and more active at first, but soon the thallus becomes meristematic throughout. As the thallus approaches its maximum size at this stage, a zone of cells extending across the base of the broadened portion remains more meristematic, and the cells above this may be slightly elongated.

Sometimes all of the cells of the germ tube are involved in the broadening of the thallus; more commonly one, and less frequently two, three, or four nearest the spore remain undivided for a time (fig. 12). With maturity of the thallus this proximal group of cells divides further in two planes to form a zone one cell thick, one to

four cells broad (usually two), and one to eight cells long (usually four to six). The cell nearest the spore may remain undivided. These cells become much elongated, and are no doubt efficient in the conduction of materials absorbed by the rhizoids (fig. 14).

Oil cells begin to form on the expanded portion of the thallus when this region is six to eight cells broad (fig. 12). They are formed almost exclusively in the marginal row of cells, certain of which cut off a small cell from the end or outer corner. Such a small cell is prevaillingly triangular, although frequently wedge-shaped or rectangular. Shortly after its formation, a large oil drop of irregular outline appears within it, and often almost fills it. Although surrounding cells may continue to divide, oil cells are not capable of further division.

The primary rhizoid continues to elongate until it is two to six times the length of the plant from spore to tip of thallus.

Secondary rhizoids take their origin from the elongated cells below the meristematic zone. The first ones appear nearest the spore, and the later ones progressively at more distal points; none has been seen to arise from the meristematic zone or from the regions beyond. In number they vary up to eight. Their method of formation is characteristic. The proximal portion of a lateral wall of a cell bulges out and forms an outgrowth which becomes a cylinder of uniform diameter (fig. 15). The chloroplasts are lost early, and both cell and rhizoid remain colorless (fig. 16). The secondary rhizoids have the same diameter as the primary rhizoid, and soon approach its length. They also have a thickened, rounded end wall, are smooth, of uniform diameter, and have no cross walls. As the chloroplasts are lost, scattered oil drops appear, the protoplasm becomes granular, especially at the tip, and the nucleus is found a short distance back from it (fig. 16).

When the primary thallus has reached its maximum size, therefore, the plant consists of the following parts: the spore; the primary rhizoid, which extends in one direction; the base of the original germ tube, which extends in the opposite direction; a long narrow band of elongated cells from some of which extend the secondary rhizoids; and a broadened unistratose region containing oil cells at its margin and remaining meristematic at its base (fig. 14). The

growth of the primary thallus stage up to this point was found to require two to three weeks. The empty spore wall remains attached until the primary thallus is mature. By the time it becomes broken off it appears to be partially decomposed, is swollen, and is more transparent than before germination. Strong sunlight is often detrimental to the thallus after the filamentous stage, rendering it stunted and broad. In weak light the thallus may be long and ribbon-shaped.

Among the abnormal shapes observed were those in which the distal portion of the thallus was twisted at a sharp angle to the proximal portion, the median line of the two portions being continuous and straight. This was probably due to a change of orientation with regard to light.

Many observations have been made by other workers on the primary thallus stage of various species of *Riella*. On the whole their interpretations agree, the main controversy being centered on the presence or absence of an apical cell. That an apical cell is present near the tip of the thallus was believed by HOFMEISTER (8), and LEITGEB (10) did not consider the growing region intercalary. HOWE and UNDERWOOD (9), GOEBEL (5), SOLMS (13, 14), and PORSILD (12) find no apical cell, but instead either consider the entire broadened portion of the thallus meristematic, or else recognize an intercalary meristematic region. DOUIN (4) figures the primary thallus stage without showing cells, and without description. The latter investigator, as well as the others, gives descriptions of the primary thallus of the several species which correspond rather closely to that of *R. americana*.

Lateral outgrowth and differentiation of stem and wing

When the cultures were three to five weeks old, growth became localized at one or both sides of the margin of the intercalary region of growth. Usually the cells on one side and at the middle mature, while those at the opposite margin remain meristematic. If only the middle cells mature and both margins remain meristematic, however, twin plants result (fig. 19).

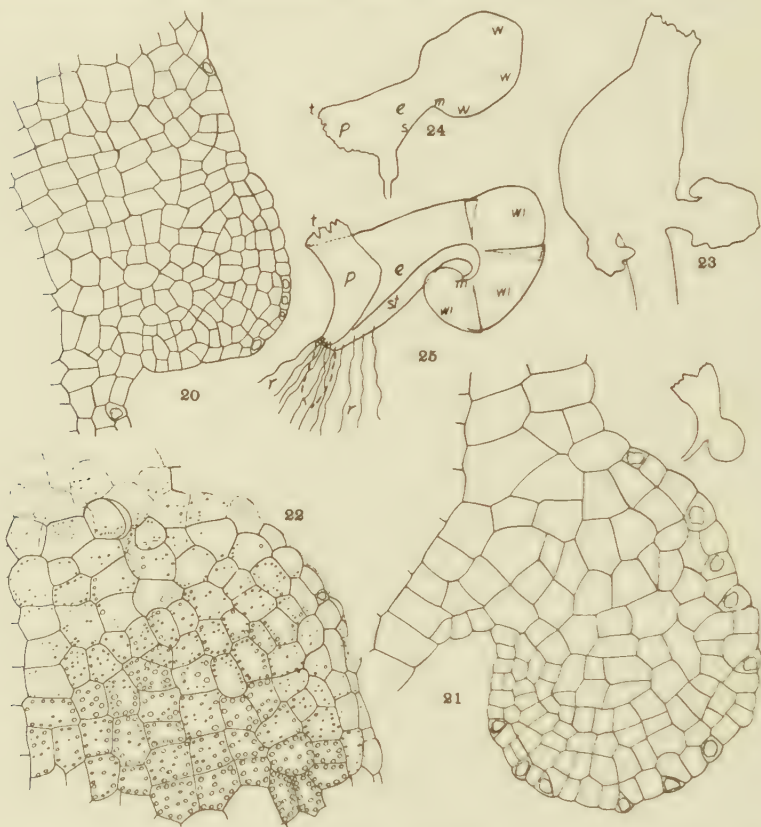
There is generally no indication of an apical cell along the meristematic margin, but sometimes a single large cell with small plastids, close to one or two oil cells, functions as an apical cell (fig. 17).

Even in these instances, however, the condition is purely temporary, the apical cell soon giving way to a number of small, actively dividing cells, which constitute a more permanent growing region (fig. 18). The cells of this region are the smallest ones of the thallus, except the oil cells. The smallest and most active of the meristematic group are those closest to the base of the thallus, that is, nearest the median line (proximal with reference to the spore). Walls come in irregularly at any angle, so that orientation of one cell bears no definite relation to that of any other, except that all cells lie in the same plane. The marginal cells, however, form a more definite row.

The new mass of tissue grows downward and outward so as to form an angle of 45° – 60° with the elongated cells below the meristematic zone, and it maintains throughout a position in the same plane as the primary thallus. A semicircular mass of tissue is soon formed (figs. 19, 20), which later becomes an almost circular outgrowth one cell in thickness (fig. 21). The cells of the adjacent primary thallus become somewhat distorted. As lateral growth continues, the primary thallus is bent to one side until it may occupy a position at right angles to its original axis, and the new growth also extends out more nearly at right angles to the original axis of the thallus (fig. 21). The cells at the base of the new growth elongate and carry the meristematic zone some distance from the primary thallus. At the same time an overgrowth occurs just beyond, on the proximal side (that is, toward the spore). This overgrowth extends around the tip of the meristematic region and back toward the primary thallus, nearly parallel with the axis of the new outgrowth, on its distal side (away from the spore). This results in the lateral outgrowth having a falcate structure which is similar, in miniature, to the shape of the adult plant, the most active cells being in the notch of the overgrown portion, and the cells between the notch and the primary thallus showing a tendency toward elongation (figs. 24, 25). Oil cells at first are not so numerous in the region of active growth, but appear on the margin of the falcate area some distance from the growing zone.

During progress of the lateral outgrowth, the primary thallus undergoes further changes. The cells at its tip lose their chloroplasts, protoplasmic granules become fewer, the cells separate at the cor-

ners and sides and come to overlap each other, their lumina finally become empty, and the walls break down and become detached



FIGS. 20-25.—Fig. 20, later stage of lateral outgrowth; $\times 145$. Fig. 21, circular stage of lateral outgrowth; primary thallus bent to one side and partially resorbed; large figure $\times 210$. Fig. 22, resorption at tip of primary thallus; $\times 145$. Fig. 23, abnormal secondary development of primary thalli from original primary thallus (one shows single lateral growing region, the other shows two); fig. 24, juvenile thallus shortly before differentiation of stem and wing; $\times 32$. Fig. 25, juvenile thallus after stem and wing have become differentiated (diagrammatic): *e*, region of elongated cells; *m*, region of most active division; *p*, primary thallus; *r*, secondary rhizoids; *s*, region destined to become stem; *st*, stem; *t*, resorbed tip of primary thallus; *w*, region destined to become wing; *wi*, wing; $\times 13$.

from the remainder of the thallus (fig. 22). This breakdown of the cells continues progressively from the tip toward the base of the

primary thallus. It usually begins at the same time as the formation of the lateral outgrowth, but it has been observed earlier. It continues until, by the time the stem and wing are differentiated, two-thirds or three-fourths of the primary thallus is broken down. The process is interpreted as being a phenomenon of resorption with the passage of the resorbed materials to the new growing region.

In place of the lateral outgrowth just discussed, a second thallus of the same type as the primary one may occasionally develop from the lateral meristematic region; such a thallus in its turn develops a slender stalklike portion and a broadened group of cells, at the base of which one or two lateral meristematic zones develop, just as on the primary thallus (fig. 23).

Under the cultural conditions discussed, the lateral outgrowth developed slowly, many of the plants died, and many others were destroyed during examination. The cultures also became overcrowded with algae, which were detrimental to the growth of *Riella*. It was further found that the narrow portion at the base of the primary thallus was easily broken off. It has been possible, to date, to grow only a single specimen to the stage of showing clearly the differentiation of stem and wing; this specimen was found 80 days after the cultures were sown (fig. 25).

The stem or axis appears on the proximal edge of the lateral outgrowth, that is, on the edge nearer the spore, and extends from the meristematic zone at the notch of the lateral outgrowth to the base of the primary thallus. This is accomplished by division of these marginal cells, which are already elongated, in the third plane to form an axis which is circular or oval in cross-section. This is the first time in the life history of *Riella* that cell divisions normally take place in the third plane. The falcate structure already described as extending from the growing point over the crest and back to the primary thallus constitutes the wing. The stem, therefore, is on the proximal margin of the lateral outgrowth (toward the spore), and the wing on the distal side (away from the spore). There is no sharp line of demarcation between the primary thallus and the new outgrowth, both of which lie in the same plane. The boundary line can be determined roughly by the fact that the cells at the base of

the new growth are somewhat larger than those of the adjacent primary thallus.

The plant at this stage is 2.6 mm. long from the base of the lateral outgrowth to the crest of the wing, and the maximum width of the wing is 0.44 mm. Since the margin of the wing has elongated more rapidly than the stem, it is a much folded and wavy structure, but shows no evidence of spiral twisting around the stem, as was at first indicated for *R. helicophylla*. The stem is 0.07 mm. wide at its distal curved region near the meristematic zone, and 0.18 mm. along most of its course, which is straight; along this straight portion it is five to seven cells wide. A portion of the primary thallus is still attached, and new rhizoids have developed from the base of the stem. There is no indication of sex organs, but trichomic outgrowths have made their appearance in the region of the growing point.

The early workers with *Riella* were much confused by the lateral outgrowth from the primary thallus and by the differentiation of the stem and wing, and their interpretations have differed materially. HOFMEISTER (8) calls attention to a lateral growing point, but he apparently thought of a growing region nearer the apex of the primary thallus; no apical cell is mentioned. He did not succeed in growing the sporelings to the point of stem and wing differentiation. TRABUT (15) does not discuss this stage, but his figures show what seems to be the primary thallus with a lateral outgrowth. GOEBEL (5) had no sporelings available. HOWE and UNDERWOOD (9) did not succeed in growing sporelings beyond the early primary thallus stage; they thought, apparently incorrectly, that the upper portion of the primary thallus becomes the wing and the lower portion the stem. They thought that the stem in *R. affinis* was being formed as a result of divisions in the third plane at the base of the primary thallus; their illustrations, however, show small, lateral, intercalary outgrowths comparable with those found by the writer for *R. americana*, although no interpretation was made of these. SOLMS (13) found two lateral outgrowths at the base of the primary thallus; one of these continues to grow while the other is suppressed. He found the growing region in the new growth, but thought that its entire lower portion becomes the stem. He maintained that an apical cell is formed later, but that, in spite of its position, it cannot be con-

sidered intercalary. His plants, however, did not actually reach the stem stage. PORSILD (12) found the lateral outgrowth from the primary thallus, and its thickening to form the stem on the side nearest the spore, but he found no apical cell at any stage of development, not even after the stem and wing had been clearly differentiated. He also reported the presence of oil cells on the primary thallus, but found less regularity in their shape than is reported in the present paper. He, too, found that the cells of the primary thallus die and empty their contents after differentiation of stem and wing. A sharp boundary line is reported to exist between the primary thallus and the wing. GOEBEL (6) found one or two growing regions localized at lateral intercalary points. He found no sharp line of demarcation between the primary thallus and the wing, hence he does not consider the lateral outgrowth (stem and wing) as a new structure, different from the primary thallus, as was thought by SOLMS and PORSILD. He found no definite wedge-shaped apical cell in the meristematic region at the tip of the stem.

Symmetry

The peculiar position of the wing with respect to the stem has been the subject of much discussion. A part of the dispute over the question of symmetry has been based on conclusions from meager information. It has been pointed out that many of the workers had no sporelings at their disposal, or else did not succeed in growing them to a sufficiently adult stage to show the origin of the stem and wing. Furthermore, the terms symmetry, dorsal and ventral, and distal and proximal, have not always been clearly defined.

In the filamentous stage, the *Riella* sporeling is evidently radially symmetrical, all sides of the short filament having the same appearance. The factor which determines the angle of the first longitudinal wall in the filament has not been determined; probably it will be found to be light or gravity.

The primary thallus stage of *R. americana* is certainly bilaterally symmetrical; that is, a plane extending through the middle of the thallus, perpendicular to it, and from its distal point to its proximal region, divides it into halves which image each other. This bilateral symmetry remains until the lateral outgrowth begins to develop on

one side; or, in the case of development of lateral outgrowths simultaneously on both sides of the primary thallus, bilateral symmetry is evident for a much longer time. There is no reason for considering the flattened, unistratose primary thallus dorsiventral, if by that term is meant that there is a difference between the two faces, for the exposed faces of the cells are alike on both sides of the thallus.

Other species of *Riella* exhibit the same condition in the primary thallus stage, as indicated by the descriptions and figures of HOFMEISTER (8), GOEBEL (5, 6), HOWE and UNDERWOOD (9), SOLMS (13), and PORSILD (12). A difference may exist in those species which grow horizontally on the substrate. Furthermore, GOEBEL (6, 7) drew a close parallel between the primary thallus of *Riella* and the gemma of *Marchantia* or *Lunularia*: in both of these cases there are one or two lateral intercalary growing regions from which the adult thalli arise; both gemma and primary thallus grow vertically at first; and the two flattened surfaces of both gemma and primary thallus are alike, the two faces of the gemma becoming different only after this structure has been in contact with the substrate for a time.

Confusion in the terminology of symmetry is mostly in evidence in connection with the lateral outgrowth, the young plant with differentiated stem and wing, and the adult plant. HOFMEISTER (8) compared the adult plant with the thallus of *Marchantia* in which one of the wings has been suppressed. LEITGEB (10) considers the wing a dorsal outgrowth of the stem, comparable with a comb standing at the tip of the stem; such a conception, however, implies that the stem develops before the wing, which is not the case. GOEBEL (5) recognizes some similarities to the thallus of *Marchantia*, but places emphasis on the fact that *Riella* grows in an erect position from the beginning (his "Profilstellung"). In a later work (7) he states that the apparently radial thallus is in reality an extraordinary modification of the usual dorsiventral thallus of the liverworts. Those species with a creeping, horizontal stem differ from the usual form of the liverworts only in that the wing is developed in a vertical position. The relationship between the wing and stem is similar in the species which grow in an erect position. He therefore considers the wing as dorsal to the stem. HOWE and UNDERWOOD consider the adult plant bilaterally symmetrical in the plane of the wing,

which is dorsal in relation to the stem. CAVERS (3) considers the wing dorsal and the stem ventral.

In the discussion of the lateral outgrowth and the adult plant of *R. americana* which follows, an effort is made to clarify the terminology of symmetry. The lateral outgrowth of the American species is from the beginning irregular, the cells nearest the spore (proximal) being smaller than those farther away (distal). The shape of the outgrowth is not perfectly semicircular, nor later absolutely circular; no plane at right angles to its surface will divide it into parts which image each other. The same is true for the older plant, in which stem and wing have been differentiated, and also for the mature plant. If the lateral outgrowth or the adult plant can be considered bilaterally symmetrical at all, it must be in the plane of the wing, as noted by HOWE and UNDERWOOD. Dorsiventrality, as just defined, is not exhibited at any stage in the development from the beginning of the lateral outgrowth to the maturity of the plant, for the two faces of the young lateral outgrowth, as well as of the stem and wing of the older plants, are alike.

As the lateral outgrowth develops, the angle which it forms with the primary thallus varies greatly; but as its development continues, a simple bending by differential growth on two opposite sides of the stem results in the adult plant being erect or ascending. In nature the mature *R. americana* is a much branched plant; only a few of the branches are definitely vertical, while most of them are ascending at various angles from 45° to 90° with the substrate. It is therefore logical to discard the terms dorsal and ventral, as used by most workers with *Riella*, who consider the wing dorsal and the stem ventral. A better terminology is to consider the wing distal with regard to the stem, using the position of the spore as a point of reference.

Summary

1. Spores of *Riella americana* were germinated in water and studied mainly in the living condition. There is little or no swelling of the spore, but oil drops are present in great numbers and a large vacuole forms at a position opposite the point of rupture.

2. The spore ruptures at the middle of the outer face, and a germ tube containing great numbers of oil drops emerges through the

spore wall, leaving a rounded opening. By apical growth a filamentous stage is produced, consisting of four to seven cells, which soon show numerous chloroplasts. The primary rhizoid originates from the germ tube between the spore and the nearest cross wall.

3. Beginning with the apical cell, most of the cells of the filament divide by longitudinal and cross divisions to form a broadened primary thallus one cell in thickness, the base of which remains as a meristematic zone. The secondary rhizoids develop as extensions of some of the cells between the spore and the broadened primary thallus. All rhizoids are smooth, colorless, and of uniform diameter.

4. The meristematic zone becomes localized at one or both sides, and one or two lateral outgrowths result which are one cell thick and in the same plane as the primary thallus, the tip of which is resorbed. The distal portion of the lateral outgrowth (away from the spore) becomes the wing of the adult plant, and the proximal margin (toward the spore) thickens by cell divisions in the third plane to form the stem. By a bending of the axis of growth the adult plant becomes erect or ascending.

5. The filamentous stage is radially symmetrical, and the primary thallus stage bilaterally symmetrical. The later stages, from the beginning of the lateral outgrowth to the adult plant, are not dorsiventral and are bilaterally symmetrical only in the plane of the wing. The wing is simply considered distal to the stem, using the position of the spore as a point of reference.

The writer wishes gratefully to acknowledge the help of Dr. W. J. G. LAND during the progress of the work and in the preparation of the manuscript.

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[Accepted for publication December 16, 1930]

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